EFFECT OF HYDRODYNAMIC PRESSURE PROCESSING AND AGING ON SARCOPLASMIC PROTEINS OF BEEF STRIP LOINS*

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ABSTRACT

*This study evaluated the effects of hydrodynamic pressure processing (HDP) and aging on the sarcoplasmic proteins of beef strip loins. Loins (*n = *12) were halved at 48 h postmortem and assigned to HDP or control treatments. Following treatment, each half was divided into three portions for aging (0, 5 or 8 d). Samples were removed for Warner-Bratzler shear force (WBSF) determination and sarcoplasmic protein isolation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis demonstrated that aging significantly influenced the composition of the sarcoplasmic protein fraction and that HDP influenced protein bands corresponding to 143, 65, 44, 36 and 19 kDa. Changes in sarcoplasmic protein composition were significantly correlated to WBSF (*r = *–0.58 to 0.45). Sarcoplasmic protein solubility decreased with both HDP (*$P < 0.05$*) and aging (*$P < 0.0001$*). Changes in solubility were significantly correlated to SDS-PAGE band intensities (*r = *–0.53 to 0.60). Data suggest that HDP and aging cause changes to sarcoplasmic proteins that may be indicators of proteolysis and tenderization.*

PRACTICAL APLICATIONS

Results from this study demonstrate that postmortem aging and hydrodynamic pressure processing of beef influences the sarcoplasmic protein profile of the muscle. The relationships between these changes and Warner-

* Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

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Bratzler shear force measurements suggest that sarcoplasmic protein changes may be potentially useful as tenderness indicators.

INTRODUCTION

Muscle tenderness represents an economically important meat quality trait that is biologically complex and difficult to control. Despite years of research, many of the underlying mechanisms that influence meat tenderness are not fully understood. Postharvest techniques such as aging and hydrodynamic pressure processing (HDP) can be used to enhance muscle tenderness and improve the quality and uniformity of less desirable cuts of meat. Postmortem aging tenderization is associated with the degradation of key myofibrillar and cytoskeletal proteins such as titin, nebulin, desmin, filamin and troponin-T by endogenous proteases (Taylor *et al.* 1995; Huff-Lonergan *et al.* 1996). HDP is a novel postharvest technique that tenderizes meat using high-energy shock waves through water. HDP has been shown to cause immediate tenderness improvements and to enhance aging tenderization (Solomon *et al.* 1997; Solomon *et al.* 2002).

The influence that tenderization techniques have on tenderness and other eating qualities of meat is due to their effects on muscle proteins. There is a lack of data, however, characterizing the influence of HDP on muscle proteins related to meat quality when combined with postmortem aging. The effects of HDP and aging on tenderness-related myofibrillar proteins are reported in a companion paper (Bowker *et al.* 2008). Much research, especially regarding postmortem aging, has focused on the role that myofibrillar and cytoskeletal proteins play in meat tenderness, but less data is available on the direct or predictive role that sarcoplasmic proteins may play in muscle tenderness. In regards to HDP, it is known that the high-energy shock waves disrupt the sarcomere ultrastructure (Zuckerman and Solomon 1998) and cause a shift in the protein content between the myofibrillar and sarcoplasmic fractions of muscle (Spanier and Romanowski 2000), but the specific effects of HDP on the protein characteristics of the sarcoplasmic fraction are not known. Thus, the objective of the current study was to investigate the effects of HDP and aging on the sarcoplasmic proteins of fresh, boneless beef strip loins using SDS-PAGE analysis.

MATERIALS AND METHODS

Muscle Samples

Twenty-four boneless strip loins were obtained from a commercial beef processing facility from Select, Yield Grade 2 and 3 beef carcasses at 24 h

postmortem. After removal of the sirloin portion, one 2.5-cm thick steak was removed from the anterior end of each strip loin for baseline determination of Warner-Bratzler shear force (WBSF). The twelve strip loins selected for use in this experiment had shear force values ranging from 6.77 to 12.84 kgf at 24 h postmortem.

Experimental Design

Strip loins were processed on four separate days with three strip loins per replication. At 48 h postmortem (designated as experimental day 0), each strip loin was split into anterior and posterior halves, which were assigned either control or HDP treatments such that each loin served as its own control. Following treatments (HDP and control) on day 0, each half strip loin was split into thirds which were assigned to either 0, 5 or 8 days of aging. All samples were then vacuum-packaged and stored at 4C for the designated aging period. Following aging, one 2.5-cm thick steak was removed from each section for WBSF determination, and a second 2.5-cm thick steak was removed for protein analysis. To account for inherent variation in tenderness along the length of the strip loins, a completely balanced design was used such that each treatment-aging combination was applied to each anatomical position along the length of the strip loins an equal number of times. Steaks used for protein analysis were trimmed free of fat, knife minced and thoroughly mixed prior to sampling in order to account for the inherent variation within a steak. The effects of the treatments on WBSF and myofibrillar proteins are reported in another paper (Bowker *et al.* 2008).

HDP Treatments

The parameters of the HDP treatment were set according to the findings of past studies described in Solomon *et al.* (2006). Strip loin sections (15 × 17.5 × 6.3 cm, 2.0–2.2 kg) designated for HDP treatment were individually packaged in boneguard bags (Cryovac©/Sealed Air Corp., Duncan, SC), briefly heat shrunk (~87C) and placed onto a 1.3-cm thick flat metal disk inside a 98-L plastic container filled with water (4–6C). The container was suspended 25 cm above the floor and a 100-g rectangular shaped binary explosive was detonated 31 cm above the meat to generate the high-energy shock waves. One sample was treated per HDP process.

Sarcoplasmic Protein Solubility (SPS)

SPS was measured using the procedure of Schilling *et al.* (2002) with modifications. Solubility was determined by homogenizing duplicate 1 g muscle samples with 10 mL of ice-cold 0.025 M potassium phosphate (pH

7.2) buffer in four 4-sec bursts with a Kinematica polytron (Model PT 10/35, Brinkman Instruments, Inc., Westbury, NY). The homogenate was incubated overnight at 4C and then centrifuged at 2,600 × g for 30 min. The supernatant was decanted and protein concentration was measured using the biuret method (Gornall *et al.* 1949). Values are expressed as mg protein per g of muscle tissue.

Sarcoplasmic Protein Isolation

The sarcoplasmic protein fraction was isolated according to the procedure of Spanier and Romanowski (2000). Trimmed muscle samples were first minced for 5 sec in a handheld blender (MR 370, Braun, Inc., Woburn, MA). Approximately 5 g of minced muscle was homogenized with 45 mL of ice-cold buffer (0.05 M Tris-HCl, 1.5 mM dithiothreitol, 1.5 mM EDTA, 1 mM sodium azide, pH 7.0) using a Waring Blendor™ equipped with a rheostat (Powerstat™, Superior Electric Co., Bristol, CT) at a setting of 65% for 10 sec. The homogenate was filtered through two layers of cheese cloth and centrifuged at 10,800 × g for 15 min (4C). The pellet was discarded and the supernatant was centrifuged at 48,200 × g for 30 min (4C). The final supernatant (sarcoplasmic protein fraction) was then assayed for protein content using the biuret method and the concentrations were adjusted to 4.0 mg/mL.

Gel Sample Preparation and SDS-PAGE Analysis

One volume of each sarcoplasmic sample was then mixed with one volume of sample buffer/tracking dye solution (8 M urea, 2 M thiourea, 0.05 M Tris (pH 6.8), 75 mM DTT, 3% SDS, 0.05% bromophenol blue), heated in boiling water for 5 min, cooled on ice and stored at −80C until analysis. For evaluation of the sarcoplasmic protein profile (Fig. 1), denatured samples were loaded (20 µg/lane) onto pre-cast 10-20% acrylamide gradient Tris-HCl gels. Gels were run in duplicate on the Bio-Rad Criterion gel system (Bio-Rad Laboratories, Hercules, CA) at a constant voltage (200 V) for approximately 1 h at 4C. The running buffer was 25 mM Tris (pH 8.3), 192 mM glycine, 2 mM EDTA and 0.1% (w/v) SDS. Gels were stained in a solution of 0.05% Coomassie brilliant blue R-250, 40% (v/v) methanol and 7% (v/v) glacial acetic acid, and destained in 15% (v/v) methanol and 7% (v/v) glacial acetic acid. Stained gel images were captured using an imaging system (Kodak Gel Logic 200; Eastman Kodak Co., Rochester, NY), and Kodak 1D Image Analysis software was used to measure the density of protein bands. Broad range (6.5 to 200 kDa) molecular weight standards (Bio-Rad Laboratories, Hercules, CA) were run on each gel to determine protein band molecular weights and to account for gel to gel variations. To account for slight variations in protein loading, the density of protein bands were expressed as a

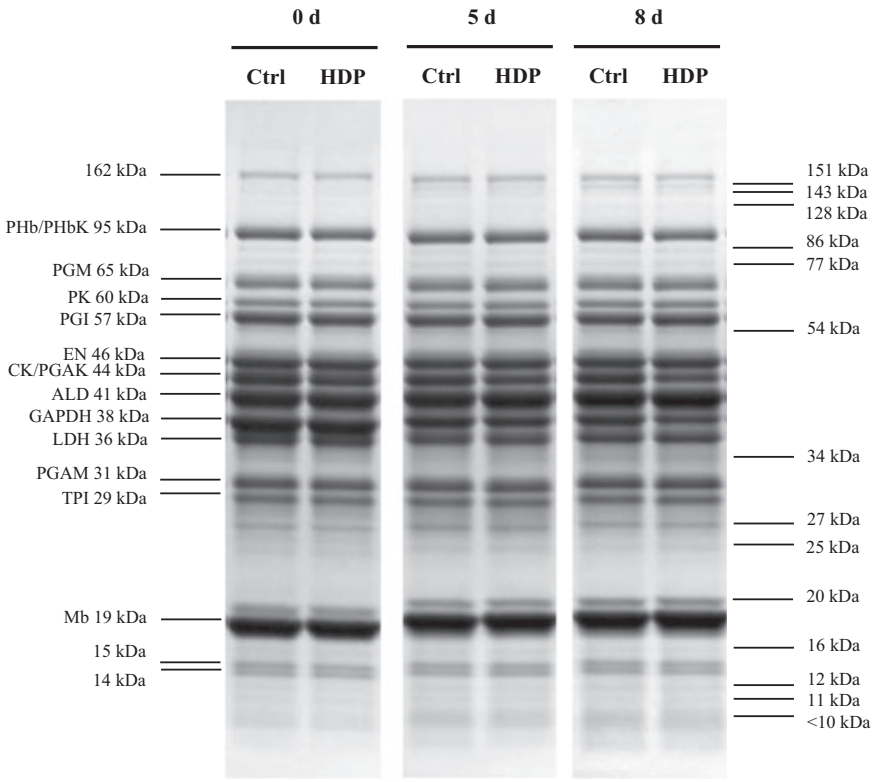

FIG. 1. GRADIENT (10–20%) SDS-PAGE GELS OF SARCOPLASMIC PROTEINS FROM CONTROL AND HDP BEEF STRIP LOINS AGED 0, 5 AND 8 DAYS
PHb, phosphorylase *b*; PHbK, phosphorylase *b* kinase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; PGAK, phosphoglycerate kinase; ALD, aldolase; GAPDH, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase; Mb, myoglobin.

percentage of the total density of all the protein bands within the lane. Due to potential protein to protein variation in Coomassie staining, comparisons were only made within a given protein band across samples.

Statistical Analysis

Data were analyzed as a 2-way ANOVA using the PROC MIXED procedure of SAS® (version 9.1, SAS Institute Inc., Cary, NC, 2002–2003). For the analysis of all parameters, the model included treatment (control or HDP), aging (0, 5 or 8 d) and the treatment by aging interaction as fixed effects and

strip loin as a random block effect. The anatomical position of each treatment-aging combination within each strip loin and the half (anterior or posterior) that was assigned HDP treatment were analyzed and found to have no significant effects on the dependent variables measured. Thus, the experimental design adequately accounted for inherent variations along the length of the strip loins and these terms were not included in the final model. Significant differences ($P < 0.05$) between means were separated with the PDIFF option. Correlation coefficients were determined using the PROC CORR procedure.

RESULTS

Protein Solubility

The effects of HDP and aging treatments on the SPS of beef strip loins are shown in Table 1. Both treatment and aging significantly influenced solubility, but a significant treatment by aging interaction effect was not detected. HDP caused a slight decrease ($P < 0.05$) in solubility compared to controls across all aging periods. Solubility decreased ($P < 0.0001$) between 0 and 5 d of aging and again from 5 to 8 d of aging in both HDP and control samples. The overall decrease in SPS from 0 to 8 d of aging was approximately 14% in all samples.

SDS-PAGE analysis of sarcoplasmic proteins

Figure 1 is a representative gel depicting the effects of HDP and aging on the SDS-PAGE profiles of sarcoplasmic proteins. The analysis of the treatment

TABLE 1.
SARCOPLASMIC PROTEIN SOLUBILITY* OF CONTROL AND HDP BEEF STRIP LOINS AGED 0, 5 AND 8 DAYS

Trt	Aging period (d)			Mean‡	*P* value§		
	0	5	8		Trt	Aging	Trt*aging
Ctrl	68.4	64.6	60.0	64.3[a]	<0.05	<0.0001	NS
HDP	67.0	61.6	59.2	62.6[b]			
Mean†	67.7[a]	63.1[b]	59.6[c]				

* Values expressed as mg protein per g of muscle tissue.
† Mean solubility within each aging period. [abc] Means with different superscripts differ significantly ($P < 0.05$).
‡ Mean solubility within each treatment. [ab] Means with different superscripts differ significantly ($P < 0.05$).
§ NS indicates not significant ($P > 0.05$).
HDP, hydrodynamic pressure processing.

effects on band intensities are shown in Table 2. Only the 44-kDa (CK/PGAK) and 143-kDa bands exhibited a significant HDP main effect. The intensity of the 44-kDa (CK/PGAK) band decreased ($P < 0.05$) with HDP treatment compared to controls but was not significantly influenced by aging. The intensity of the 143-kDa band, which was absent in the majority of day 0 samples, was higher ($P < 0.05$) in HDP samples after 5 and 8 d of aging. Similarly, the 151-kDa band was not detectable in day 0 samples but the intensity of the band increased ($P < 0.0001$) from 5 to 8 d of aging and was numerically higher in controls than HDP-treated samples.

The majority of the protein bands did not exhibit significant HDP effects or HDP by aging interaction effects, but were influenced by aging (Table 2). Bands corresponding to 95 (PHb/PHbK), 57 (PGI), 38 (GAPDH), 31 (PGAM), 29 (TPI), 27, 25, 20 and 15 kDa decreased ($P < 0.05$) in intensity with aging. Conversely, bands corresponding to 128, 60 (PK), 54, 41 (ALD), 34, 12 and <10 kDa increased ($P < 0.05$) in intensity with aging. The intensity of the 162 and 77 kDa bands increased from 0 to 5 d of aging, but decreased slightly from 5 to 8 d of aging.

Three protein bands exhibited a significant treatment by aging interaction effect (Table 2). The intensity of the 36-kDa (LDH) band increased with aging in the HDP-treated samples but did not significantly change in the controls. Similarly, there was an aging-related increase in the intensity of the 19-kDa (Mb) band in HDP samples but not in control samples. The intensity of the 65-kDa (PGM) band did not significantly change with aging in control samples. In the HDP-treated samples, however, the intensity of the 65-kDa (PGM) band was the highest after 5 d of aging.

Bands corresponding to 86, 46 (EN), 16, 14 and 11 kDa were not influenced by aging or HDP treatments.

Correlation of Sarcoplasmic Protein Changes to Tenderness and Solubility

The correlation between WBSF measurements and SDS-PAGE protein band intensities are shown in Table 3. Ten of the protein bands were correlated to WBSF measurements across all treatments and aging periods. WBSF measurements were positively correlated ($r = 0.22$ to 0.45) to the intensities of the 95- (PHb/PHbK), 46- (EN), 38- (GAPDH), 27-, and 20-kDa bands. WBSF measurements were negatively correlated ($r = -0.25$ to -0.58) to the intensities of the 151-, 143-, 41- (ALD), 12- and <10-kDa bands. Table 3 also shows the correlation between protein band intensities and WBSF within control and HDP samples. The intensities of the 151-, 95- (PHb/PHbK), 60- (PK), 54-, 41- (ALD), 38- (GAPDH) and 12-kDa bands were more highly correlated to WBSF in controls than in HDP-treated samples. Conversely, the intensities of

TABLE 2.
SDS-PAGE* ANALYSIS OF SARCOPLASMIC PROTEINS ISOLATED FROM CONTROL AND HDP BEEF STRIP LOINS AGED 0, 5 AND 8 DAYS

Protein band	Trt	Aging period (d)			Mean‡	*P* value§		
		0	5	8		Trt	Aging	Trt × aging
162 kDa	Ctrl	0.39	0.72	0.64	0.58			
	HDP	0.37	0.76	0.57	0.57	NS	<0.0001	NS
	Mean†	0.38^{c}	0.74^{a}	0.61^{b}				
151 kDa	Ctrl	ND	0.07	0.35	0.21			
	HDP	ND	0.01	0.29	0.15	NS	<0.0001	NS
	Mean†	–	0.04^{b}	0.32^{a}				
143 kDa	Ctrl	ND	0.25	0.13	0.19^{b}			
	HDP	ND	0.32	0.19	0.26^{a}	<0.05	<0.0001	NS
	Mean†	–	0.28^{a}	0.16^{b}				
128 kDa	Ctrl	0.09	0.17	0.18	0.15			
	HDP	0.05	0.25	0.20	0.17	NS	<0.0001	NS
	Mean†	0.07^{b}	0.21^{a}	0.19^{a}				
95 kDa PHb/PHbK	Ctrl	4.61	4.52	4.08	4.41			
	HDP	4.52	4.53	3.85	4.30	NS	<0.0001	NS
	Mean†	4.57^{a}	4.52^{a}	3.97^{b}				
86 kDa	Ctrl	0.42	0.45	0.41	0.43			
	HDP	0.38	0.58	0.36	0.44	NS	NS	NS
	Mean†	0.40	0.52	0.39				
77 kDa	Ctrl	0.36	0.60	0.47	0.48			
	HDP	0.33	0.70	0.45	0.49	NS	<0.0001	NS
	Mean†	0.35^{b}	0.65^{a}	0.46^{b}				
65 kDa PGM	Ctrl	3.80^{xyz}	4.10^{wx}	3.91^{wxy}	3.94			
	HDP	3.64^{yz}	4.18^{w}	3.52^{z}	3.78	NS	<0.0001	<0.05
	Mean†	3.72	4.14	3.72				
60 kDa PK	Ctrl	2.75	2.98	2.90	2.87			
	HDP	2.72	2.95	2.82	2.83	NS	<0.0001	NS
	Mean†	2.73^{b}	2.97^{a}	2.86^{a}				
57 kDa PGI	Ctrl	5.82	5.69	5.60	5.70			
	HDP	5.74	5.65	5.37	5.59	NS	<0.01	NS
	Mean†	5.78^{a}	5.67^{ab}	5.48^{b}				
54 kDa	Ctrl	1.94	2.17	2.21	2.11			
	HDP	1.99	2.23	2.21	2.14	NS	<0.05	NS
	Mean†	1.96^{b}	2.20^{a}	2.21^{a}				
46 kDa EN	Ctrl	8.38	8.44	8.04	8.29			
	HDP	8.46	8.25	7.93	8.21	NS	NS	NS
	Mean†	8.42	8.35	7.98				
44 kDa CK/PGAK	Ctrl	8.13	8.17	7.94	8.08^{a}			
	HDP	8.04	7.52	7.75	7.77^{b}	<0.05	NS	NS
	Mean†	8.08	7.84	7.85				
41 kDa ALD	Ctrl	9.44	10.54	10.62	10.20			
	HDP	9.80	10.30	11.77	10.63	NS	<0.01	NS
	Mean†	9.62^{b}	10.42^{ab}	11.20^{a}				
38 kDa GAPDH	Ctrl	9.81	8.89	7.88	8.86			
	HDP	9.92	8.25	7.57	8.58	NS	<0.0001	NS
	Mean†	9.87^{a}	8.57^{b}	7.72^{c}				
36 kDa LDH	Ctrl	5.95^{z}	5.78^{z}	5.70^{z}	5.81			
	HDP	5.97^{z}	5.81^{z}	6.30^{y}	5.93	NS	<0.05	<0.01
	Mean†	5.96	5.80	6.00				

TABLE 2. CONTINUED

Protein band	Trt	Aging period (d)			Mean‡	*P* value§		
		0	5	8		Trt	Aging	Trt × aging
34 kDa	Ctrl	2.61	3.13	3.27	3.00			
	HDP	2.68	3.29	3.26	3.08	NS	<0.0001	NS
	Mean†	2.65^{b}	3.21^{a}	3.26^{a}				
31 kDa PGAM	Ctrl	7.66	7.24	7.11	7.34			
	HDP	7.75	7.22	6.84	7.27	NS	<0.0001	NS
	Mean†	7.71^{a}	7.23^{b}	6.97^{b}				
29 kDa TPI	Ctrl	5.35	4.47	4.50	4.77			
	HDP	5.33	4.36	4.56	4.75	NS	<0.0001	NS
	Mean†	5.34^{a}	4.41^{b}	4.53^{b}				
27 kDa	Ctrl	2.43	2.19	1.78	2.13			
	HDP	2.51	2.37	1.64	2.17	NS	<0.0001	NS
	Mean†	2.47^{a}	2.28^{a}	1.71^{b}				
25 kDa	Ctrl	1.49	1.32	1.16	1.32			
	HDP	1.56	1.61	1.16	1.44	NS	<0.05	NS
	Mean†	1.52^{a}	1.46^{ab}	1.16^{b}				
20 kDa	Ctrl	2.65	2.24	2.39	2.43			
	HDP	2.68	2.28	2.23	2.39	NS	<0.0001	NS
	Mean†	2.66^{a}	2.26^{b}	2.31^{b}				
19 kDa Mb	Ctrl	10.37^{y}	10.49^{x}	10.42^{xy}	10.43			
	HDP	10.26^{z}	10.50^{x}	11.03^{w}	10.60	NS	NS	<0.05
	Mean†	10.31	10.50	10.73				
16 kDa	Ctrl	0.84	0.73	0.82	0.80			
	HDP	0.87	0.79	0.83	0.83	NS	NS	NS
	Mean†	0.85	0.76	0.83				
15 kDa	Ctrl	1.50	1.23	1.39	1.37			
	HDP	1.49	1.34	1.35	1.39	NS	<0.05	NS
	Mean†	1.49^{a}	1.28^{b}	1.37^{b}				
14 kDa	Ctrl	1.50	1.36	1.51	1.45			
	HDP	1.45	1.48	1.40	1.44	NS	NS	NS
	Mean†	1.47	1.42	1.46				
12 kDa	Ctrl	0.06	0.43	0.60	0.36			
	HDP	0.08	0.61	0.58	0.42	NS	<0.0001	NS
	Mean†	0.07^{b}	0.52^{a}	0.59^{a}				
11 kDa	Ctrl	0.61	0.51	0.56	0.56			
	HDP	0.59	0.61	0.58	0.59	NS	NS	NS
	Mean†	0.60	0.56	0.57				
<10 kDa	Ctrl	0.83	1.17	1.46	1.15			
	HDP	0.75	1.40	1.49	1.21	NS	<0.0001	NS
	Mean†	0.79^{b}	1.28^{a}	1.48^{a}				

* Values represent the least square means of the intensity of the SDS-PAGE bands expressed as a percentage of the total intensity of all bands in the sample.

† Mean intensity of bands within each aging period. ^{abc} Mean intensities with different superscripts differ significantly ($P < 0.05$).

‡ Mean intensity of bands within each treatment. ^{ab} Mean intensities with different superscripts differ significantly ($P < 0.05$).

§ NS indicates not significant ($P > 0.05$).

^{wxyz} Mean intensities of bands with different superscripts differ significantly ($P<0.05$).

ND indicates that bands were not detected in gel.

PHb, phosphorylase *b*; PHbK, phosphorylase *b* kinase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; PGAK, phosphoglycerate kinase; ALD, aldolase; GAPDH, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase; Mb, myoglobiny; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

TABLE 3.
CORRELATIONS BETWEEN SDS-PAGE BAND INTENSITIES OF SARCOPLASMIC PROTEINS AND WARNER-BRATZLER SHEAR FORCE OF BEEF STRIP LOINS

Protein band	Pearson correlation coefficients of band intensity and WBSF		
	Across all samples ($n = 72$)	Control samples ($n = 36$)	HDP samples ($n = 36$)
162 kDa	–0.01	–0.05	0.01
151 kDa	–0.33**	–0.46**	–0.34*
143 kDa	–0.25*	–0.26	–0.21
128 kDa	–0.02	0.11	–0.08
95 kDa (PHb/PHbK)	0.22*	0.29*	0.15
86 kDa	0.10	0.22	0.04
77 kDa	0.02	0.08	0.02
65 kDa (PGM)	0.16	0.11	0.09
60 kDa (PK)	–0.12	–0.30*	–0.04
57 kDa (PGI)	–0.15	–0.25	–0.22
54 kDa	–0.15	–0.33*	–0.16
46 kDa (EN)	0.25*	0.16	0.45**
44 kDa (CK/PGAK)	0.08	0.13	0.01
41 kDa (ALD)	–0.46****	–0.55***	–0.42*
38 kDa (GAPDH)	0.45****	0.54***	0.39*
36 kDa (LDH)	–0.16	–0.12	–0.15
34 kDa	–0.15	–0.15	–0.09
31 kDa (PGAM)	0.14	0.08	0.20
29 kDa (TPI)	0.05	0.08	0.02
27 kDa	0.36**	0.44**	0.42*
25 kDa	0.17	0.22	0.32*
20 kDa	0.25*	0.16	0.39*
19 kDa (Mb)	–0.13	0.02	–0.20
16 kDa	0.17	0.18	0.24
15 kDa	0.14	0.29	0.07
14 kDa	0.09	0.15	0.05
12 kDa	–0.41***	–0.47**	–0.39*
11 kDa	–0.13	–0.04	–0.13
<10 kDa	–0.58****	–0.68****	–0.63****

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.
WBSF, Warner-Bratzler shear force; PHb, phosphorylase *b*; PHbK, phosphorylase *b* kinase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; PGAK, phosphoglycerate kinase; ALD, aldolase; GAPDH, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase; Mb, myoglobin.

the 46- (EN), 25- and 20-kDa bands of HDP samples exhibited higher correlations with WBSF than controls.

The relationship between day 0 SDS-PAGE profiles and WBSF after 0, 5,and 8 d of aging is shown in Table 4. The 151- and 143-kDa bands were not

TABLE 4.
CORRELATIONS BETWEEN SDS-PAGE BAND INTENSITIES OF SARCOPLASMIC PROTEINS MEASURED AT DAY 0 AND WARNER-BRATZLER SHEAR FORCE MEASURED AFTER 0, 5 AND 8 DAYS AGING IN BEEF STRIP LOINS

Protein band	Pearson correlation coefficients of day 0 band intensity and WBSF		
	WBSF at day 0 ($n = 24$)	WBSF at day 5 ($n = 24$)	WBSF at day 8 ($n = 24$)
162 kDa	0.31	0.34	0.33
151 kDa	Band not detectable in day 0 samples		
143 kDa	Band not detectable in day 0 samples		
128 kDa	0.51**	0.42*	0.56**
95 kDa (PHb/PHbK)	0.26	0.27	0.32
86 kDa	–0.07	–0.12	–0.03
77 kDa	0.27	0.16	0.34
65 kDa (PGM)	0.32	0.41*	0.37*
60 kDa (PK)	0.02	0.04	0.01
57 kDa (PGI)	–0.35	–0.23	–0.15
54 kDa	–0.26	–0.24	–0.16
46 kDa (EN)	0.44*	0.45*	0.47*
44 kDa (CK/PGAK)	0.05	–0.20	–0.37
41 kDa (ALD)	–0.12	0.14	0.15
38 kDa (GAPDH)	–0.32	–0.16	–0.14
36 kDa (LDH)	–0.11	–0.23	–0.41*
34 kDa	0.10	0.01	–0.05
31 kDa (PGAM)	–0.01	–0.17	–0.10
29 kDa (TPI)	–0.13	–0.14	–0.07
27 kDa	0.00	–0.13	–0.10
25 kDa	–0.17	–0.33	–0.30
20 kDa	0.20	0.24	0.22
19 kDa (Mb)	–0.13	–0.06	–0.15
16 kDa	0.05	–0.16	–0.15
15 kDa	–0.14	0.01	0.04
14 kDa	–0.13	0.16	0.11
12 kDa	–0.21	–0.22	–0.24
11 kDa	–0.23	–0.32	–0.26
<10 kDa	–0.44*	–0.36*	–0.37*

* $P < 0.05$; ** $P < 0.01$

WBSF, Warner-Bratzler shear force; PHb, phosphorylase *b*; PHbK, phosphorylase *b* kinase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; PGAK, phosphoglycerate kinase; ALD, aldolase; GAPDH, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase; Mb, myoglobin.

observed in day 0 samples; therefore, the correlation between day 0 band intensity and WBSF at 0, 5 and 8 d of aging was not determined. The WBSF measured after each aging period was significantly correlated to the day 0 intensities of the 128-, 46- (EN) and <10-kDa bands. The day 5 and 8 WBSF

measurements were positively correlated to the intensity of the 65-kDa (PGM) band at day 0, while the WBSF at day 8 was also negatively correlated to the intensity of the 36-kDa (LDH) band at day 0.

The correlation between SPS and SDS-PAGE protein band intensities are shown in Table 5. Thirteen of the protein bands were correlated to SPS measurements across all treatments and aging periods. SPS measurements were positively correlated ($r = 0.28$ to 0.60) to the intensities of the 95- (PHb/PHbK), 44- (CK/PGAK), 38- (GAPDH) and 20-kDa bands. SPS measurements were negatively correlated ($r = -0.22$ to -0.53) to the intensities of the 151-, 60- (PK), 41- (ALD), 36- (LDH), 19- (Mb), 14-, 12-, 11- and <10-kDa bands. Table 5 also shows the correlation between protein band intensities and SPS within control and HDP samples. The intensities of the 151-, 95- (PHb/PHbK), 86-, 60- (PK) and 20-kDa bands were more highly correlated to SPS in controls than in HDP-treated samples. Conversely, the intensities of the 41- (ALD), 38- (GAPDH), 11- and <10-kDa bands of HDP samples exhibited higher correlations with SPS than controls.

DISCUSSION

Meat quality attributes are influenced by the inherent characteristics of muscle proteins. This study was conducted to determine the effects of HDP and aging on the tenderness and related muscle protein changes in beef strip loins. The tenderness measurements of the beef strip loins from this study are reported in the companion paper (Bowker *et al.* 2008). It was shown that HDP decreased WBSF 23% compared to controls at 0, 5 and 8 d of aging. Furthermore, aging decreased WBSF approximately 20% from 0 to 5 d and another 11% from 5 to 8 d in both HDP and control samples. The effects of HDP and aging on myofibrillar proteins are reported in the companion paper (Bowker *et al.* 2008). The current study investigated the effects of HDP and aging treatments on the sarcoplasmic protein fraction of the muscle.

The sarcoplasmic protein component of muscle tissue consists of water-soluble proteins within the cytoplasm of muscle cells and constitutes 30–35% of the total muscle protein. The isolation procedure used in the present study resulted in protein samples primarily consisting of the cytoplasmic fraction, which includes myoglobin and the glycolytic enzymes. Since muscle tenderization is often accompanied by myofibrillar and cytoskeletal protein degradation (Taylor *et al.* 1995; Huff-Lonergan *et al.* 1996), it is possible that the protein isolates in the current study contain soluble fragments of myofibrillar proteins in addition to sarcoplasmic proteins. Using SDS-PAGE analysis, Kolczak *et al.* (2003) observed that the abundance of high molecular weight proteins (205–2800 kDa) in centrifugal drip from beef muscle increased with

TABLE 5.
CORRELATIONS BETWEEN SDS-PAGE BAND INTENSITIES OF SARCOPLASMIC PROTEINS AND SARCOPLASMIC PROTEIN SOLUBILITY (SPS) OF BEEF STRIP LOINS.

Protein band	Pearson correlation coefficients of band intensity and SPS		
	Across all samples ($n = 72$)	Control samples ($n = 36$)	HDP samples ($n = 36$)
162 kDa	0.04	–0.01	0.09
151 kDa	–0.33**	–0.37*	–0.29
143 kDa	–0.14	0.01	–0.27
128 kDa	0.07	0.18	0.04
95 kDa (PHb/PHbK)	0.60****	0.66****	0.54***
86 kDa	0.22	0.32*	0.11
77 kDa	–0.04	0.04	–0.11
65 kDa (PGM)	–0.06	0.02	–0.14
60 kDa (PK)	–0.40***	–0.48**	–0.35*
57 kDa (PGI)	0.04	0.28	–0.21
54 kDa	–0.14	–0.11	–0.19
46 kDa (EN)	–0.02	–0.13	0.12
44 kDa (CK/PGAK)	0.57****	0.56***	0.58***
41 kDa (ALD)	–0.42***	–0.38*	–0.46**
38 kDa (GAPDH)	0.47****	0.44**	0.49**
36 kDa (LDH)	–0.33**	–0.34*	–0.32*
34 kDa	0.06	0.14	–0.03
31 kDa (PGAM)	–0.03	–0.22	0.14
29 kDa (TPI)	0.04	–0.01	0.07
27 kDa	0.17	0.13	0.22
25 kDa	0.21	0.17	0.28
20 kDa	0.28*	–0.47**	–0.09
19 kDa (Mb)	–0.36**	–0.35*	–0.36*
16 kDa	–0.09	–0.21	0.03
15 kDa	–0.04	–0.01	–0.07
14 kDa	–0.22*	–0.17	–0.27
12 kDa	–0.44****	–0.45**	–0.42**
11 kDa	–0.40***	–0.29	–0.50**
<10 kDa	–0.53****	–0.36*	–0.68****

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.
SPS, sarcoplasmic protein solubility; PHb, phosphorylase *b*; PHbK, phosphorylase *b* kinase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; PGAK, phosphoglycerate kinase; ALD, aldolase; GAPDH, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglycerate mutase; TPI, triosephosphate isomerase; Mb, myoglobin.

postmortem aging. It was speculated that these proteins were products of titin degradation. Pospiech *et al.* (2000) confirmed the presence of titin degradation products in the centrifugal drip of pig muscles using immunoblotting. Similarly, Xiong and Anglemier (1989) observed an aging-related appearance

of 100-kDa and 500-kDa proteins in the sarcoplasmic fractions of beef muscle that was thought to be of myofibrillar origin. Thus, past research supports the presence of proteins of myofibrillar origin in the isolated water-soluble protein components of muscle.

The non-structural functions of sarcoplasmic proteins and the fact that they are characteristically soluble *in situ* suggests that alterations to these proteins would have little direct impact on muscle tenderness. Ultrastructural studies have shown that tenderness is related to the degree of fragmentation within and between myofibrils, regardless if it is caused by enzyme-induced proteolysis as with aging (Davey and Dickson 1970; Taylor *et al.* 1995; Ho *et al.* 1996) or by physical disruption due to high-energy shock waves as with HDP (Zuckerman and Solomon 1998). Thus, changes in the composition or inherent properties of the sarcoplasmic protein fraction are thought to be indicators of proteolysis and tenderization rather than playing a direct structural role in muscle tenderness.

In the present study, SDS-PAGE techniques were utilized to characterize treatment-related changes in the composition of the sarcoplasmic protein samples. Diminishing band intensities due to either aging or HDP treatment were hypothesized to be the direct result of protein degradation and/or the loss of protein solubility. Increasing band intensities with either aging or HDP treatment were hypothesized to be the result of the accumulation of a protein degradation product and/or an increase in the extractability for that particular protein.

Overall, aging had a greater effect on the sarcoplasmic protein composition than did HDP treatment. This is not surprising given that postmortem aging is characterized by extensive proteolysis and HDP tenderization is associated with the physical disruption of the sarcomere structure. Aging-related changes in bands corresponding to 95 kDa (PHb/PHbK) and 38 kDa (GAPDH) confirm past findings regarding the degradation of sarcoplasmic proteins. Using SDS-PAGE analysis of sarcoplasmic proteins, Okayama *et al.* (1992) reported that phosphorylase b (PHb), creatine kinase (CK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gradually decreased during the postmortem conditioning of beef. GAPDH has also been shown to degrade in pork muscle stored at refrigerated temperatures for 15 d (Okumura *et al.* 2003). Additionally, the aging-related decrease in the 25- and 27-kDa bands in the current study is similar to the aging-related decrease in the abundance of 26-28 kDa protein bands observed in the centrifugal drip fraction of beef by Kolczak *et al.* (2003).

Some of the bands observed in the protein profiles were not consistent across all samples. In approximately half of the samples, several bands ranging from 200 to 210 kDa were visually observed in day 0 samples and the intensity of these bands seemed to diminish with aging. Additionally, a diffuse protein band approximately 28 kDa was observed after 8 d of aging

in both control and HDP samples. The relatively low staining intensity of these bands and their absence from some of the samples prevented quantification for analysis purposes. The absence or extremely low abundance of 151-, 143- and 128-kDa bands in day 0 samples and their subsequent appearance with aging suggests that these bands are potentially degradation products of larger muscle proteins. Similarly, the aging-related increase in the intensities of low molecular weight bands (12 kDa and <10 kDa) suggest that they also may be fragments resulting from the degradation of larger proteins. Further research is needed, however, to determine the exact identity and origin of these protein bands.

Data from the current study indicate that HDP treatment may influence the degradation or extractability of several proteins present in the sarcoplasmic fraction of muscle. HDP treatment influenced the 143-, 65- (PGM), 44- (CK/PGAK), 36- (LDH) and 19-kDa (Mb) bands either directly or through an interaction with aging. HDP directly increased the intensity of the 143-kDa band which was hypothesized to be the breakdown product of a larger protein. The lower abundance of the 44-kDa band in HDP-treated samples compared to controls indicates that HDP enhances the breakdown of this protein. The susceptibility of the 44-kDa protein to degradation is not surprising given that creatine kinase (44 kDa) is thought to be one of the most labile sarcoplasmic proteins in beef (Scopes 1964). The significant correlations between the intensities of the 44-, 36- and 19-kDa bands and protein solubility suggest that HDP effects on SDS-PAGE sarcoplasmic protein bands may be related to changes in protein extractability. Overall, these data, for the first time, indicate that HDP has a direct influence on sarcoplasmic protein degradation.

Many of the changes observed in the composition of the sarcoplasmic protein samples by SDS-PAGE analysis were found to be associated with changes in tenderness (Table 3). WBSF values were most highly correlated to the 41- (ALD), 38- (GAPDH), 12- and <10-kDa bands. Overall, the sarcoplasmic protein composition was more highly correlated to WBSF in control samples. This is likely the result of the decreased variation in the WBSF measurements of HDP-treated samples compared to controls. To decipher the potential usefulness of sarcoplasmic protein composition as a predictor of tenderness, the correlation measurements between day 0 SDS-PAGE profiles and WBSF at 0, 5 and 8 d were determined (Table 4). The data demonstrated that in a few cases, protein band intensities at day 0 were significantly correlated to WBSF after 5 and 8 d of aging. Others have also investigated the correlation between sarcoplasmic protein composition and tenderness. Using capillary electrophoresis, Patel *et al.* (2006) observed that aging-related changes in the abundance of 30- and 100-kDa proteins from the extracted sarcoplasmic fractions and drip proteins from beef strip loins were correlated to WBSF. Lametsch *et al.* (2003) found a significant correlation between

triosephosphate isomerase (TPI) and WBSF in pork muscle. Despite the fact that sarcoplasmic proteins probably do not play a role in determining tenderness, the data from the current study suggest that changes in sarcoplasmic protein profiles may reflect increased proteolysis and might be useful as potential markers for tenderness development.

In the current study, protein solubility was measured as an indicator of the direct effects that HDP and aging had on the chemical nature of sarcoplasmic proteins. The solubility of most globular proteins, like many of those found in the sarcoplasmic fraction of muscle, is primarily determined by the interaction between the aqueous solvent and the charged and polar groups on the surface of the proteins. Thus, changes in SPS suggest changes in protein chemistry. Spanier and Romanowski (2000) suggested that changes in protein solubility may have been responsible for the observation that HDP treatment caused a shift in the protein distribution between the sarcoplasmic and myofibrillar fractions of muscle upon differential centrifugation. The finding that HDP caused a slight decrease in SPS in the current study suggests that shifts in the protein distribution between the sarcoplasmic and myofibrillar components may be related to changes in protein solubility.

The current study demonstrated that SPS diminished with muscle aging. Given the heterogeneity of the sarcoplasmic protein fraction in muscle, it is likely that changes in the composition of the protein profiles influenced overall solubility. It is also unlikely that changes in solubility were equal across all proteins in the sarcoplasmic fraction. Although it does not signal a cause and effect relationship, the observation that thirteen protein bands in the SDS-PAGE profiles were correlated to overall solubility indicate that changes in profile composition may influence solubility (Table 5). The influence of composition on the solubility of the sarcoplasmic fraction, however, seems to vary between control and HDP-treated samples. Thus, solubility data from the current study suggest that both HDP and aging have direct effects on the inherent chemical properties of sarcoplasmic proteins.

For most of the variables measured in this study, there was not a significant treatment interaction. HDP and aging effects on SPS were additive. Except for a few protein bands in the SDS-PAGE profile, there was not a significant interaction between HDP and aging treatments. Overall, data from the current study indicate that there is no synergism between HDP and aging regarding their impact on sarcoplasmic protein characteristics.

CONCLUSION

Data from this study indicate that aging and HDP influence the composition and solubility of the sarcoplasmic protein fraction of beef muscle. While

treatment-induced alterations to the sarcoplasmic protein characteristics were found to be correlated to WBSF, sarcoplasmic protein changes were thought to be indicators of proteolysis rather than having a direct structural impact on tenderness.

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